Memoirs of the Museum of Victoria 56(2):543–555 (1997)

A BIOCHEMICAL TAXONOMIC STUDY OF SPINY CRAYFISH OF THE GENERA *ASTACOPSIS* AND *EUASTACUS* (DECAPODA: PARASTACIDAE) IN SOUTH-EASTERN AUSTRALIA

LYNDA AVERY AND CHRISTOPHER M. AUSTIN[1]

School of Aquatic Science and Natural Resources Management, Deakin University, PO Box 423, Warrnambool, Vic. 3280, Australia
[1]Author for correspondence

Abstract

Avery, L. and Austin, C.M., 1997. A biochemical taxonomic study of spiny crayfish of the genera *Astacopsis* and *Euastacus* (Decapoda : Parastacidae) in south-eastern Australia. *Memoirs of the Museum of Victoria* 56(2): 543–555.

A study of allozyme variation amongst spiny crayfish species of the genera *Astacopsis* (Huxley) and *Euastacus* Clark in south-eastern Australia was carried out to evaluate the current morphologically-based taxonomy. Nineteen populations representing ten putative species were analysed for variation at 32 allozyme loci. Heterozygosities were found to be low and typical for parastacid crayfishes. Significant geographical variation in allelic frequencies was found only in *E. yarraensis*. Analysis of genetic relationships amongst samples provided unambiguous support for the recognition for five *Euastacus* species *(E. armatus, E. kershawi, E. diversus, E. neodiversus* and *E. woiwuru)* and two species of *Astacopsis (A. gouldi* and *A. franklinii)*. Support for the recognition of *A. tricornis* is equivocal as a sample of this species displayed a relatively low level of allozymic divergence from a sample of *A. franklinii*. Samples of *E. yarraensis* and *E. bispinosus* could not be distinguished from *E. armatus* indicating the need for more detailed taxonomic studies of this complex of species.

Introduction

Freshwater crayfish belonging to the genera *Astacopsis* (Huxley) and *Euastacus* Clark, commonly called spiny crayfish, are widespread in eastern Australia. *Euastacus* species occur only on mainland Australia in the states of Queensland, New South Wales, Victoria and South Australia, whereas *Astacopsis* species are found only in Tasmania (Clark, 1936; Riek, 1969). Both genera have a preference for cool, pristine and well-oxygenated freshwater environments and inhabit mostly permanent rivers, streams, lakes and impoundments. Spiny crayfish are found in both highland and lowland country in the cooler southern part of Australia but are restricted to more elevated and isolated areas in the northern part of their distribution (Swain et al., 1982; Morgan, 1983, 1986, 1988; Horwitz, 1990a; Hamr, 1992).

Several species of spiny crayfish are capable of growing to very large sizes. *Astacopsis gouldi* is the largest freshwater crayfish in the world and is known to reach sizes in excess of 3 kg (Olszewski, 1980) however, animals exceeding 2 kg are rarely caught today (Horwitz, 1990a). Several *Euastacus* species, including *E. armatus* (von Martens), *E. kershawi* (Smith) and *E. bispinosus* Clark from southern Australia are able to grow to weights in excess of 2 kg. As a consequence of their large size all of these crayfish species have attracted considerable attention from amateur fishermen. The lower numbers of spiny crayfish being caught and their decreasing size over recent years have been attributed to the combination of increased recreational fishing pressure, habitat alteration and the slow growth of these species (Campbell, 1990; Honan and Mitchell, 1995). Conservation concerns have led to the implementation of a range of fishing regulations (Horwitz, 1990a; Anon, 1991; Lindermans and Rutzou, 1991) for *Euastacus* and *Astacopsis* species. In addition to fishing pressure, spiny crayfish also appear to be highly vulnerable to habitat change; significant range reductions have been recorded for several species and a number of species are now listed as rare or vulnerable (Horwitz, 1990a; Honan and Mitchell, 1995).

Studies of the biology and ecology of spiny crayfish species of both genera are scant (Clark, 1937; Hamr, 1992; Honan and Mitchell, 1995). However, in contrast, the taxonomy of these crayfish has been comprehensively examined by several authors in recent years (Riek, 1969; Swain et al., 1982; Morgan, 1983, 1986, 1988; Hamr, 1992) using classical morphologically-based approaches. These studies have lead to major taxonomic rearrangements and, at times, contradictions indicating that morphological

variation is extensive and complex within these crayfish. Thus, despite these recent taxonomic studies there are still doubts concerning the number and identity of species within both *Astacopsis* and *Euastacus* (see taxonomic history).

According to the biological species concept, species consist of groups of individuals potentially capable of exchanging genetic material with each other and producing viable offspring, but are reproductively isolated from other such groups (Mayr, 1963). While this definition has conceptual merits it is difficult to put into practice as studies aimed at the direct identification of reproductive groups are demanding and rarely undertaken by taxonomists. Further, even when reproductive studies are conducted the interpretation of results is often equivocal. An advantage of using biochemical and molecular genetic techniques to address taxonomic questions is that they can be used to indirectly establish or infer reproductive relationships amongst populations (Richardson et al., 1986) thus providing information consistent with the biological definition of species. In addition, a number of studies have shown biochemical data to be very useful in resolving species boundaries where morphological variation is difficult to interpret (Richardson et al., 1986).

Australian freshwater crayfish are a taxonomically difficult group and the technique of allozyme electrophoresis has proven useful in the delineation of species boundaries in the genera *Cherax* (Austin, 1986, 1996; Campbell et al., 1994; Austin and Knott, 1996) *Engaeus* (Horwitz et al., 1990) and *Grainastacus* (Zeidler and Adams, 1990). The aim of this project was to extend these biochemical taxonomic studies of Australian parastacid crayfish to spiny crayfish by evaluating the morphologically-based taxonomy of the genus *Astacopsis* and Victorian species of the genus *Euastacus* using allozyme gel electrophoresis.

Taxonomic history

The first taxonomic record of an Australian freshwater crayfish was a spiny crayfish described by Shaw in 1794 as *Cancer serratus*. This species was subsequently placed in the genus *Astacopsis*, erected by Huxley (1878), who placed the genus within the newly described family, the Parastacidae, which accommodated all the crayfish from the southern hemisphere. Since this early work there have been two major revisions of the family Parastacidae, by Clark (1936) and Riek (1969). Clark (1936) divided the spiny crayfish into two genera, *Astacopsis* and *Euastacus*. She restricted *Astacopsis* to the Tasmanian spiny crayfish to which she added two new species, giving a total of three species for this genus. At the same time she erected a new genus, *Euastacus*, for the Australian mainland spiny crayfish which at that time contained 11 species. In a subsequent revision of *Euastacus* Clark (1941) retained only four of these previously described species, elevated two from subspecies and described three new species thereby recognising nine species in the genus.

The next major contributor to the taxonomy of spiny crayfish was Riek (1956, 1969). Riek (1956) split *Euastacus* by erecting the genus *Euastacoides* for several small species which have restricted distributions in northern New South Wales and southern Queensland. In his major revision of Australian parastacid crayfish (Riek, 1969) he retained the three spiny crayfish genera and described an additional species for *Astacopsis*, bringing the number of species within this genus up to four. He also described a number of new species of mainland spiny crayfish bringing the total number of *Euastacus* species up to 27.

A relatively recent revision of *Astacopsis* by Swain et al. (1982) reduced the species number from four to two, however Hamr (1992) re-established the three species originally described by Clark (1936). The most recent revisions of *Euastacus* have been by Morgan (1983, 1986, 1988, 1989) who undertook a comprehensive and detailed review of *Euastacus* throughout its distribution which resulted in the synonymy of *Euastacoides* with *Euastacus* and the recognition of a total of 37 species within the redefined genus which included 16 newly described species (Morgan, 1983, 1986, 1988, 1989; Horwitz, 1995).

Materials and methods

Sample collection

The majority of *Euastacus* and *Astacopsis* specimens were collected during the day from rivers and streams in state parks and on private land. Collection techniques consisted of the use of baited strings, drop nets in rivers and deep streams and the turning over of rocks in shallow streams. The specimens of *E. neodiversus* from Tarwin River West were dug from burrows in the river bank. Wherever possible each species examined in this study was sampled from the

Table 1. OTU code, sample size (n) and collecting locality for each population of *Euastacus* species and *Astacopsis* species sampled in Victoria and Tasmania.

Species	OTU	n	Locality
Victoria			
E. kershawi	EK-a	1	Shady Creek, NE of Warragul, Vic.
E. kershawi	EK-b	1	Tarra River, Yarram, Vic.
E. bispinosus	EB-a	2	Crawford River, Dirk Dirk, Vic.
E. neodiversus	EN-a	2	Dingo Creek, N of Welshpool, Vic.
E. neodiversus	EN-b	2	Turtons Creek, S of Mirboo, Vic.
E. neodiversus	EN-c	4	Tarwin River West, Vic.
E. woiwuru	EW-a	2	Olinda Creek, Olinda, Vic.
E. woiwuru	EW-b	1	Sassafras Ck., Monbulk, Vic.
E. woiwuru	EW-c	3	Dandenong National Park, Vic.
E. yarraensis	EY-a	4	Stephensons Falls, Gellibrand River, Vic.
E. yarraensis	EY-b	2	Aire River, S. of Beach Forest, Vic.
E. yarraensis	EY-c	2	Lake Elizabeth, E Barwon River, Vic.
E. yarraensis	EY-d	1	Williamsons Ck, Ballarat, Vic.
E. yarraensis	EY-e	3	Woori Yallock Creek, Vic.
E. diversus	ED-a	1	Orbost, Vic.
E. armatus	EA-a	1	Ovens River. Harrietville, Vic.
Tasmania			
A. tricornis	AT-a	3	Arve River, Mt. Hartz road. Tas.
A. franklinii	AF-a	3	New Town rivulet, Nth. Hobart, Tas.
A. gouldi	AG-a	1	Big River, Wynard, Tas.

type locality or from other sites referred to in the literature (Clark, 1936; Morgan, 1986; Hamr, 1992). The species sampled, population codes and locality descriptions are given in Table 1. Specimens were either frozen in the field in liquid nitrogen or kept alive on ice until transferred to the laboratory.

Tissue samples

Each crayfish specimen was initially stored in a labelled polythene freezer bag at −20°C. Frozen specimens were placed in liquid nitrogen and sent to the Evolutionary Biology Unit of South Australian Museum, where they were stored at −80°C. Abdominal muscle tissue was dissected from thawed specimens and placed in a plastic vial with an equal volume of lysing solution (500 ml of distilled water containing 50 mg NADP and 0.5 ml β-mecaptoethanol). This mixture was homogenised using a Branson Sonifier (model B-12) and then centrifuged for 10 min. The supernatant was removed and stored in Micro-Haematocrit Capillary Tubes which were then held at −80°C until required. All carcasses were preserved in 7% formalin.

Electrophoresis

A pilot study carried out with *Euastacus* species using starch gel electrophoresis identified twenty enzymatic loci but using cellulose acetate gels ('Cellogel') forty three enzymatic loci could be, therefore only 'Cellogel' was used in all subsequent electrophoresis runs. However, 11 loci proved to be too difficult to score consistently across all species and so were excluded from the final analysis. The remaining 32 loci were screened for all samples and are presented in Table 2 with their abbreviations, enzyme commission (EC) numbers and details of running conditions. The general procedures for running 'Cellogel' electrophoresis are given by Richardson et al. (1986).

Zymograms were interpreted using standard approaches (Richardson et al., 1986). Putative allozymes were designated by letters in the order of mobility starting with the slowest migrating allozyme and were scored as genotypes. Allelic frequencies, heterozygosities and Nei's genetic identity (I) corrected for small sample size (Nei, 1978) were calculated using BIOSYS-1 (Swof-

Table 2. Stains and buffers used in the electrophoretic analysis of *Astacopsis* and *Euastacus* samples.

Enzyme name	Abbreviation	E.C.number[1]	No. of loci	buffer[2]
Aconitase hydratase	Acon	4.2.1.3	2	B
Aminoacyclase	Acyc	3.5.1.14	1	C
Fructose-bisphosphate aldolase	Ald	4.1.2.13	1	B
Arginine kinase	Argk	2.7.3.3	1	C
Enolase	Enol	4.2.1.11	1	B
Fructose-1, 6-diphosphatase	Fdp	3.1.3.11.	1	B
Alanine aminotransterase	Gpt	2.6.1.9	1	B
Guanine deaminase	Gda	3.5.4.3	1	C*
Lactoyl-glutathione lyase	Glo	4.4.1.5	1	C
Aspartate aminotransferase	Got	2.6.1.1	2	B
General protein	Gp	—	1	C
Glucose-6-phosphate isomerase	Gpi	5.3.1.9	1	B
Isocitrate dehydrogenase	Idh	1.1.1.42	2	B
Lactate dehydrogenase	Ldh	1.1.1.27	1	B
Malate dehydrogenase	Mdh	1.1.1.37	2	B/C
'Malic' enzyme	Me	1.1.1.40	1	B
Mannose-6-phosphate isomase	Mpi	5.3.1.8	1	B
Nucleoside-diphosphate kinase	Ndpk	2.7.4.6	1	B
Dipeptidase	Pep A	3.4.13.X	1	C
Tripeptidase aminopeptidase	Pep-B	3.4.11.X	1	A
Dipeptidase	Pep-C	3.4.13.X	1	C
Phosphoglyerate mutase	Pgam	2.7.5.3	1	B/C
Phosphogulyolate dehydrogenase	6Pgd	1.1.1.44	1	B*
Phosphoglycerate kinase	Pgk	2.7.2.3	1	C
Phosphoglucomutase	Pgm	2.7.5.1	2	C
Pyruvate kinase	Pk	2.7.1.40	1	B
Triose-phosphate isomerase	Tpi	5.3.1.1	1	B/C

[1]enzyme commission number
[2]buffers used: A=0.01M citrate-phosphate, pH 6.4
B=0.02M phosphate, pH7.0
B*=20 mg NADP and 8.5 mg $MgCl_2$ in 300 ml soaking solution
C=0.05M Tris-maleate, pH 7–8
C*=0.05M Tris-maleate, pH 7–8 with 1mM $MgCl_2$

ford and Selander, 1981) from genotypic data. A matrix of percentage fixed allelic differences was calculated for each pairwise combination of OTUs (Richardson et. al., 1986). Nei's genetic identity (I) and percentage fixed allelic differences were calculated using only the loci which stained in both OTUs. Dendrograms were constructed using the unweighted pair group method with arithmetic averages (UPGMA) technique (Sneath and Sokal, 1973) from the matrices of percentage fixed differences and Nei's genetic identity using Phylip, version 3.56 (Felsenstein, 1982).

Although it may appear that the sample sizes used in this study with respect to the number of individual crayfish per population are small, it has been established that for taxonomic applications the number of individuals from specific locations does not need to be large as long as these individuals are scored for a relatively large number of electrophoretic loci (Richardson et. al., 1986; Nei, 1978).

Results

Allelic variation amongst OTUs was recorded at 30 loci; two loci, Gpt and Ldh, were invariant (Table 3). The average estimated heterozygosities per locus (H_E), observed heterozygosities (H_O) and proportion of polymorphic loci (P) for each OTU are given in Table 4. Observed heterozygosities (H_O) for both genera are low, ranging from 0.0 to 0.078. The weighted average heterozygosities (H_O) for *Euastacus* and *Astacopsis* are 0.028 and 0.024 respectively. For each sample set H_O and H_E do not differ substantially from each other as indicated by the overlapping standard errors.

Genetic relationships amongst all populations of *Euastacus* and *Astacopsis* are summarised in Table 5 with Nei's genetic identities given in the lower diagonal and percentage fixed differences given in the upper diagonal. Figure 1 shows a dendrogram of the relationships amongst samples derived from percentage fixed differences. As the UPGMA dendrogram based upon Nei's genetic identities was very similar to Figure 1 it is not shown.

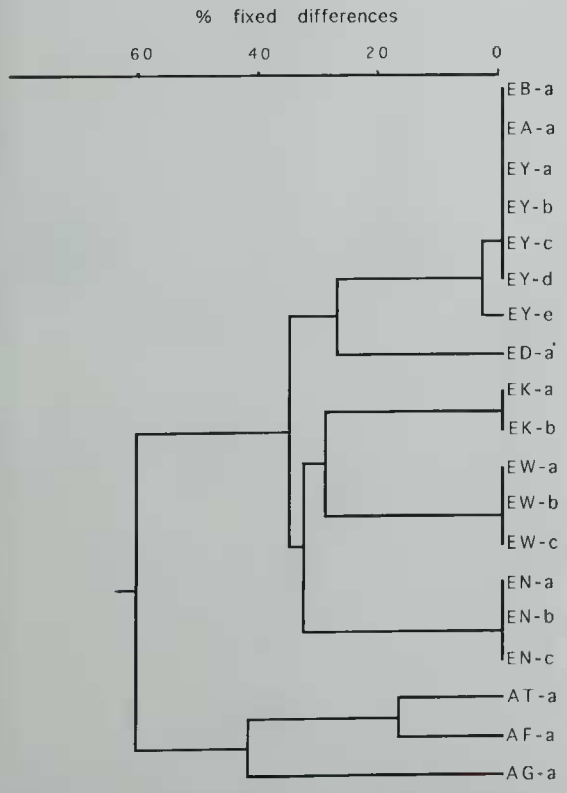

Figure 1. UPGMA dendrogram derived from a matrix of % fixed differences (see Table 1 for sample codes).

From these analyses (Table 5 and Figure 1) it can be seen that there is a primary split between the Victorian *Euastacus* species and the Tasmanian *Astacopsis* species which share an average genetic identity of I = 0.34. Amongst the three *Astacopsis* species, *A. gouldi* (AG-a) is quite distinct sharing an average genetic similarity of I = 0.51 and I = 0.52 with *A. tricornis* (AT-a) and *A. franklinii* (AF-a) respectively. Although distinct from each other, *A. tricornis* (AT-a) and *A. franklinii* (AF-a) share a much higher similarity (I=0.82) than either does with *A. gouldi* (AG-a).

From Figure 1 it can be seen that although seven putative species of *Euastacus* were examined only five distinct clusters are apparent. Four of these clusters correspond to the species *E. kershawi* (EK-a and -b), *E. diversus* (ED-a), *E. neodiversus* (EN-a, -b and -c) and *E. woiwuru* (EW-a, -b and -c) as delineated by Morgan (1986). The distinctiveness of these taxa is clearly reflected by their genetic identity values (Table 5). The genetic identity values within species are small (I = 0.97–1.00) compared with the differences amongst species (I = 0.49–0.68). In terms of fixed allelic differences, comparisons between populations within species showed no fixed differences, whereas between-species differences ranged from eight to nine fixed differences.

In contrast to these clear cut differences, the fifth cluster groups the populations of three species together, viz, *E. yarraensis* (EY-a, -b, -c, -d and -e), *E. bispinosus* (EB-a) and *E. armatus* (EA-a) which share a high degree of genetic similarity (I = 0.89–1.00). No fixed allelic differences were found amongst these three putative species. The only sample within this cluster which is even slightly divergent is the most easterly sample of *E. yarraensis* which differs by one fixed differences from the more westerly samples of this species. Thus, the western samples of *E. yarraensis*, EY-a, -b, -c and -d, are in fact more closely related to *E. armatus* and *E. bispinosus* (I = 0.98–1.00) than to the eastern sample of this species, EY-e (I = 0.91–0.92).

Discussion

Heterozygosities

The average observed heterozygosities (H_O) found in *Euastacus* and *Astacopsis* agree with the low values recorded in decapods (Tracey et al., 1975; Mulley and Latter, 1980; Nelson and Hedgecock, 1980; Hedgecock et al., 1982) and with those found previously for spiny crayfish (*E.*

Table 3. Allele frequencies at each locus. Where allele frequencies are not given the frequency is 1.00. Alleles are listed alphabetically in order of increasing distance from the cathode. Sample sizes are given below OTU codes. Asterisks indicates the failure of enzyme to stain at that particular locus.

Locus	EB-a 2	EA-a 1	EY-a 4	EY-b 2	EY-c 2	EY-d 1	EY-e 3	ED-a 1	EK-a 1	EK-b 1	EW-a 2	EW-b 1	EW-c 3	EN-a 2	EN-b 2	EN-c 4	AT-a 3	AF-a 3	AG-a 1
Acon-1	a	***	a	a	a	a	a	a	a	a	a(0.25) b(0.75)	b	b	a	a	a	c	c	c
Acon-2	a	***	a	a	a	a	a	***	a	a	a	a	a	a	a	a	a	b	***
Acyc	b	b	b	b	b	b	b	b	a(0.50) b(0.50)	a(0.50) b(0.50)	a	a	***	b	b	b	***	***	***
Ald	b	b	b	b	b	b	b	b	b	b	a	a	a	a	a	a	b(0.83) c(0.17)	b	b
Argk	b	b	b	b	b	b	b	b	b	b	b	b	b	c	c	c	a	a	b
Enol	b	b	b	b	b	b	b	b	b	b	b(0.83) d(0.17)	b	b(0.75) d(0.25)	b	b	b	a	a	c
Fdp	c	c	c	c	c	c	c	c	a	a	a(0.75) b(0.25)	a(0.50) b(0.50)	a	c	c	c	d	d	c
Gpt	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Gda	b	b	b	b	b	b	b	***	d	d	d	d	d	d	d	d	a	c	***
Glo	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	a	a	a
Got-1	b	b	b	b	b	b	b	b	b	b	c	b(0.50) c(0.50)	c	b	b	b	a	a	***
Got-2	d	d	d	d	d	d	b(0.67) d(0.33)	b(0.50) d(0.50)	b	b	c	c	c	b	b	b	a	a	b
Gp	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b	b	a
Gpi	b	b	b	b	b	b	b	b	b	b	b(0.75) c(0.25)	b	b(0.83) c(0.17)	a(0.50) b(0.50)	a(0.50) b(0.50)	b	c	b(0.33) c(0.67)	c
Idh-1	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b	b	b
Idh-2	b	b	b	b	b	b(0.50) c(0.50)	b	a	a	a	b	b	b	a	a	a	c	c	***
Ldh	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Mdh-1	b	b	b	b	b	b	a(0.67) b(0.33)	b	b	b	b	b	b	b	b	b	b	b	b
Mdh-2	b	b	b	b(0.75) c(0.25)	b	b	b(0.33) c(0.67)	a	b	b	b	b	a(0.50) b(0.50)	b	b	b	b	b	b

Table 3. Continued

Locus	EB-a 2	EA-a 1	EY-a 4	EY-b 2	EY-c 2	EY-d 1	EY-e 3	ED-a 1	EK-a 1	EK-b 1	EW-a 2	EW-b 1	EW-c 3	EN-a 2	EN-b 2	EN-c 4	AT-a 3	AF-a 3	AG-a 1
Me	c	c	c	b(0.50) c(0.50)	c	c	c	c	c	c	a	a	a	a	a(0.75) b(0.25)	a	a	a	a
Mpi	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	a
Ndpk	b	b	b	b	b	b	b	a	b	b	b	b	b	b	a(0.50) b(0.50)	a(0.13) b(0.87)	b	b	b
Pep-A	d	***	d	d	d	d	d	c	c	c	d	d	d	d	d	d	a	b	b
Pep-B	d	d	d	d	d	c(0.50) d(0.50)	d	c	a	a	b	b	b	c	c	c	f	d	e
Pep-C	a	***	a	a	a	a	a	d	c	c	b	b	b	b	b	b	***	***	***
Pgam	b	b	b	b	b	b	b	b	a	a	a(0.25) b(0.75)	a	a(0.83) b(0.17)	b	b	b	b	a(0.50) b(0.50)	b(0.50) c(0.50)
6Pgd	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a(0.87) b(0.13)	b	b	a
Pgk	c	c	c	c	c	c	c	a	a	a	a	a	a	c	c	c	b	b	b
Pgm-1	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b	b	c
Pgm-2	d	d	d	d	d	d	d	b	c	c	b	b	b	b	b	b	a	b	a
Pk	c	c	c	c	c	c	b	c	b	b	b	b	b	b	b	b	a	a	b
Tpi	b	b	b	b	b	b	b	b	b	b	b	b	b	a	a	a	b	b	b

Table 4. Sample size (n), percentage of polymorphic loci (P)[1], average estimated heterozygosities per locus (H_E) and the observed heterozygosities per locus (H_O), (standard errors in parentheses) for each OTU.

Population	n	P(%)	H_O (SE)	H_E (SE)
E. bispinosus-a	2	0.0	0.000	0.000
E. armatus-a	1	0.0	0.000	0.000
E. yarraenesis-a	4	0.0	0.000	0.000
E. yarraenesis-b	2	0.0	0.000	0.000
E. yarraenesis-c	2	6.3	0.047 (0.034)	0.036 (0.026)
E. yarraenesis-d	1	6.3	0.063 (0.043)	0.063 (0.043)
E. yarraenesis-e	3	9.4	0.000	0.050 (0.028)
E. diversus-a	1	3.1	0.031 (0.031)	0.031 (0.031)
E.kershawi-a	1	3.1	0.031 (0.031)	0.031 (0.031)
E. kershawi-b	1	3.1	0.031 (0.031)	0.031 (0.031)
E. woiwuru-a	2	15.6	0.078 (0.033)	0.078 (0.033)
E. woiwuru-b	1	6.3	0.063 (0.043)	0.063 (0.043)
E. woiwuru-c	3	12.5	0.042 (0.020)	0.050 (0.025)
E. neodiversus-a	2	3.1	0.031(0.031)	0.021 (0.021)
E. neodiversus-b	2	9.4	0.078 (0.046)	0.057 (0.032)
E. neodiversus-c	4	6.3	0.016 (0.011)	0.016 (0.011)
A. tricornis-a	3	3.1	0.010 (0.010)	0.010 (0.010)
A. franklinii-a	3	6.3	0.031 (0.023)	0.035 (0.025)
A. gouldi-a	1	3.1	0.031 (0.031)	0.031 (0.031)

[1] A locus is considered polymorphic if the frequency of the most common allele does not exceed 95%.

bispinosus and *E. armatus)* by Campbell (1990) and for land crayfish (*Engaeus* spp.) by Horwitz et al. (1990). A possible explanation for low heterozygosity levels is that the effective population sizes of spiny crayfish may fall below the number of reproducing adults required to prevent the loss of genetic diversity through inbreeding effects. This can be caused by 'bottlenecks', which are drastic reductions in population size. The effects of a severe bottleneck on heterozygosity may be extremely long lived, in the order of 10^6 generations (Hedgecock et al., 1982). The duration of bottleneck effects are extended further in species with low rates of population increase. Recent ecological studies have found that spiny crayfish are slow to reach maturity, are long lived and frequently have small population sizes (Lindermans and Rutzou, 1991; Honan and Mitchell, 1995) which means that populations of these crayfish would be both prone to bottleneck events and slow in recovering from them.

Delineation of species

Generally accepted principles for the interpretation of electrophoretic data in relation to taxonomic studies have been established (Thorpe, 1982; Richardson et al., 1986) and have been applied to parastacid crayfish species by Horwitz et al. (1990), Zeidler and Adams (1990), Campbell et al. (1994), Austin (1986, 1996) and Austin and Knott (1996). These principles set guidelines for delineating species by using fixed allelic differences or levels of genetic similarity. For example, if allopatric populations have less than 15% fixed differences or genetic identities of 0.85 or greater they are generally considered to be conspecific, conversely if populations have greater than 15% fixed differences or genetic identities less than 0.85 they are usually considered to be separate species. As the results of applying the recommendations of Richardson et al. (1986) using percent fixed difference and those of Thorpe (1982) based on genetic similarity are very similar, only the former will be discussed here on.

Using the criteria of Richardson et al. (1986) the recognition of five species of *Euastacus (E. armatus, E. kershawi, E. diversus, E. neodiversus* and *E. woiwuru)* and three species of *Astacopsis* (*A. gouldi, A. franklinii* and *A. tricornis)* is supported by the allozyme data (Table 6). The find-

Table 5. Summary of genetic relationships amongst 19 populations of spiny crayfish derived from 32 loci. Above the diagonal are given percentage fixed differences and below the diagonal are given Nei's unbiased identity (I).

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
E. bispinosus-a (1)	*	0.0	0.0	0.0	0.0	0.0	3.1	26.6	34.3	34.3	31.2	27.5	38.7	34.4	34.3	34.3	70.0	63.3	50.0
E. armatus-a (2)	1.000	*	0.0	0.0	0.0	0.0	3.5	22.2	32.1	32.1	39.2	39.2	38.7	35.7	35.7	39.2	69.2	62.5	50.0
E. yarraensis-a (3)	1.000	1.000	*	0.0	0.0	0.0	3.1	26.6	34.3	34.3	37.5	43.7	37.0	34.3	34.3	34.3	66.6	65.5	53.8
E. yarraensis-b (4)	0.994	1.000	0.994	*	0.0	0.0	3.1	26.6	34.3	34.3	37.5	40.6	38.7	34.3	31.2	34.3	66.6	65.5	53.0
E. yarraensis-c (5)	1.000	0.994	1.000	0.994	*	0.0	3.1	26.6	34.3	34.3	37.5	40.6	38.7	34.3	34.3	34.3	66.6	65.5	53.8
E. yarraensis-d (6)	0.989	0.982	0.984	0.977	0.984	*	3.1	26.6	28.1	28.1	37.5	40.6	38.7	34.3	34.3	34.3	63.3	62.5	53.8
E. yarraensis-e (7)	0.908	0.920	0.909	0.910	0.909	0.891	*	34.3	28.1	28.1	34.3	37.5	35.4	34.3	34.3	34.3	66.6	71.8	53.8
E. diversus-a (8)	0.723	0.766	0.723	0.723	0.720	0.752	0.635	*	30.0	30.0	40.0	46.6	41.3	31.2	30.0	30.0	72.4	71.8	57.6
E. kershawi-a (9)	0.649	0.667	0.646	0.634	0.646	0.656	0.663	0.678	*	0	28.1	28.1	32.2	37.5	37.5	37.5	70.0	68.7	53.8
E. kershawi-b (10)	0.655	0.667	0.651	0.604	0.651	0.662	0.669	0.678	1.000	*	28.1	28.1	32.2	37.5	37.5	37.5	70.0	68.7	53.8
E. woiwuru-a (11)	0.599	0.602	0.596	0.601	0.596	0.589	0.591	0.509	0.656	0.651	*	0.0	0.0	28.1	28.1	28.1	60.0	65.6	50.0
E. woiwuru-b (12)	0.590	0.600	0.587	0.591	0.587	0.581	0.578	0.496	0.688	0.683	0.980	*	0.0	31.2	31.2	31.2	66.6	65.6	57.6
E. woiwuru-c (13)	0.574	0.586	0.574	0.574	0.581	0.567	0.590	0.513	0.662	0.699	0.990	0.976	*	29.0	29.0	29.0	60.0	59.3	50.0
E. neodiversus-a (14)	0.656	0.633	0.647	0.652	0.647	0.674	0.665	0.646	0.636	0.642	0.687	0.674	0.667	*	0.0	0.0	66.6	68.7	50.0
E. neodiversus-b (15)	0.652	0.628	0.644	0.652	0.644	0.670	0.661	0.676	0.633	0.638	0.676	0.662	0.655	1.000	*	0.0	66.6	68.7	50.0
E. neodiversus-c (16)	0.662	0.640	0.654	0.658	0.654	0.680	0.671	0.661	0.643	0.648	0.691	0.680	0.671	0.998	0.993	*	63.3	68.7	50.0
A. tricornis-a (17)	0.330	0.329	0.330	0.330	0.328	0.352	0.293	0.248	0.296	0.296	0.350	0.307	0.304	0.305	0.285	0.304	*	16.6	42.3
A. franklinii-a (18)	0.334	0.372	0.334	0.334	0.332	0.340	0.297	0.279	0.300	0.300	0.352	0.340	0.328	0.298	0.278	0.303	0.820	*	42.3
A. gouldi-a (19)	0.408	0.484	0.408	0.408	0.407	0.412	0.434	0.353	0.427	0.427	0.434	0.392	0.391	0.412	0.393	0.402	0.521	0.519	*

ing of fixed allelic differences ranging from 22.2 to 46.6% for *Euastacus* and 16.6 to 42.3% for *Astacopsis* is similar to the findings for others parastacid crayfish by Austin (1986, 1996) and Austin and Knott (1996) for species of *Cherax* and by Horwitz et al. (1990) for species of *Engaeus*.

Of the eight species of spiny crayfish recognised above, the separation of *A. franklinii* from *A. tricornis* is the most doubtful on the basis of both allozyme and morphological evidence. These two species represent the most closely related pair of taxa recognised in this study with only 16.6% fixed differences, which is only marginally above the recommended 15% level (Richardson et al., 1986) for delineating species. Zeidler and Adams (1990) synonymised the crayfish species *Gramastacus insolitus* and *G. gracilis*, which were found to have a fixed allelic difference of 14%. Thus, the allozyme evidence supporting the recognition of *A. tricornis* is not strong based on a rigid interpretation of the genetic-yardstick approach. However, the allozyme differences between the sample of this species and the sample of *A. franklinii* are reasonably substantial given their close geographic proximity (less than 100 km) and given that geographic variation in allozyme frequencies tends to be low in spiny crayfish species (Campbell, 1990, this study)

The morphological evidence supporting the status of *A. tricornis* is also far from unequivocal. Swain et al. (1982) did not consider there were sufficient morphological differences to warrant the separation of *A. tricornis* from *A. franklinii* on the basis of an examination of variation in a range of morphological characteristics. In particular they noted that 'spininess' which can include the 'tricorn' rostral tip of *A. triconis*, increases with crayfish size. In contrast, Hamr (1992) re-established *A. tricornis* on the basis of variation in a limited number of characters associated with the rostrum, size and general spininess. Clearly, the taxonomy of these two species of *Astacopsis* needs to be clarified and would benefit from a more detailed study of both morphological and allozyme variation.

Morgan (1986) expressed some concern about the specific status of *E. woiwuru* in relation to *E. neodiversus* because he could only distinguish between them on the basis of minor differences in spination. He described them as a 'species complex', which according to Mayr's (1963) definition would warrant their recognition as subspecies. However, the results of this study clearly indicate that recognition of these two species is justified as they show a high level of electrophoretic divergence from each other.

The status of *E. diversus* is somewhat uncertain as only a very limited number of specimens have been located from a restricted geographical range in north eastern Victoria (Morgan, 1986). This study clearly distinguishes *E. diversus* from the other taxa examined in this study and worthy of the endangered species status it has been given by the Victorian Department of Conservation and Natural Resources. It will, however, be necessary to compare samples of *E. diversus* with the morphologically similar *E. bidawalus* (Morgan, 1986) to verify it is in fact a genetically distinct species. If there are no major genetic differences between these two species then both the

Table 6. Comparison between the current classification of spiny crayfish in south eastern Australia and a classfication supported by this study.

Current taxonomy	This study
E. diversus	*E. diversus*
E. kershawi	*E. kershawi*
E. woiwuru	*E. woiwuru*
E. neodiversus	*E. neodiversus*
E. armatus	*E. armatus*
E. yarraensis	*E. armatus*
E. bispinosus	*E. armatus*
A. gouldi	*A. gouldi*
A. franklinii	*A. franklinii*
A. tricornis	*A. tricornis (A. franklinii?)*

current taxonomy and the conservation status of *E. diversus* will need to be reviewed.

The most surprising finding of this study was the failure to separate *E. bispinosus* and *E. yarraensis* from *E. armatus*. Strict application of the criteria for interpreting allozyme data suggest that only one widespread and morphologically variable species, *E. armatus*, should be recognised. Morgan (1986) observed that *E. armatus* and *E. yarraensis* are morphologically similar species and, although he notes similarities between *E. bispinosus* and *E. kershawi*, he couples *E. bispinosus* with *E. armatus* and *E. yarraensis* in his taxonomic key. In fact the major differences amongst *E. armatus, E. yarraensis* and *E. bispinous* recorded by Morgan (1986) essentially relate to the degree of spination of the thorax and abdomen. Given the concerns expressed by Swain et al. (1982) and Austin and Knott (1996) on the taxonomic reliability of characters relating to 'spininess' in relation to *Astacopsis* spp. and to *Cherax* spp. respectively, the morphological evidence supporting the distinctiveness of these *Euastacus* species is not strong. A more detailed assessment of morphological variation within this complex of species is clearly warranted. This group of species would benefit from an examination of DNA variation using a more sensitive molecular genetic technique than allozyme electrophoresis for resolving fine-scale genetic differences amongst populations.

Phylogenetics

Although this study is being extended to an examination of phylogenetic relationships amongst spiny crayfish using numerical cladistic techniques (Austin and Avery, in prep.) it is worth commenting on the relationship between *Euastacus* and *Astacopsis*. Morgan (1983) considered the phylogeny of spiny crayfish using as a 'primary' character the male cuticle partition, which is found in *Astacopsis* and several species of *Euastacus* from south-eastern Victoria *(E. bidawalus, E. diversus, E. neodiversus* and *E. woiwurru)*. He considered species possessing a partition to be more closely related to each other than those lacking a partition, which supports the widening of the taxonomic definition of *Astacopsis* to encompass mainland species. Consistent with this possibility, several species of freshwater crayfish are known to occur both in northern Tasmania and the extreme south of mainland Australia (Riek, 1969; Horwitz 1988, 1990b) indicating that Bass Strait has not been a significant barrier to the dispersal of freshwater crayfish. Further, the findings by Patak and Baldwin (1984) of very few immunochemical differences in the haemocyanins between the two spiny crayfish genera is also consistent with Morgan's theory of a possible close relationship. The finding in this study of significant allozyme differences between the *Astacopsis* and *Euastacus* species and their clustering into two discrete groups (Figure 1), however, supports the present taxonomic delineation of these genera in south-eastern Australia.

Acknowledgments

This study was funded by Deakin University's Centre for Aquatic Resources Utilisation and Management and a Museum of Victoria Student Grant to the senior author. Both authors are also particularly grateful for the help and advice of Mark Adams, E.B.U., South Australian Museum in running 'cellogels'. Dr Alastair Richardson, University of Tasmania, and many other people helped in the collection of spiny crayfish specimens. Victorian crayfish were collected under permit 93/R/041 issued by the Department of Conservation and Natural Resources of Victoria and Tasmanian crayfish were collected under permit 94/29 issued by the Inland Fisheries Commission of Tasmania.

References

Anon, 1991. *Freshwater fish of Victoria. Spiny freshwater crayfish.* Department of Conservation and Environment-Fisheries Management Division. Inland Fisheries Management Branch. Victoria.

Austin, C.M., 1986. *Electrophoretic and morphological systematic studies of the genus* Cherax *(Decapoda : Parastacidae) in Australia.* Unpublished Ph. D. Thesis, University of Western Australia.

Austin, C.M., 1996. An electrophoretic and morphological taxonomic study of the freshwater crayfish genus *Cherax* Erichson (Decapoda: Parastacidae) in northern and eastern Australia. *Australian Journal of Zoology* in press.

Austin, C.M. and Knott, B., 1996. Systematics of the freshwater crayfish genus *Cherax* Erichson (Decapoda: Parastacidae) in southeastern Australia: electrophoretic, morphological and habitat variation *Australian Journal of Zoology* in press.

Campbell, N.J.H., 1990. *An electrophoretic project on* Euastacus armatus *and* Euastacus bispinosus. Unpublished report, Zoology Department, University of Adelaide.

Campbell, N.J.H., Geddes, M.C. and Adams, M., 1994. Genetic variation in yabbies *Cherax destructor* and *C. albidus* (Crustacea: Decapoda:

Parastacidae) indicates the presence of a single, highly substructured species. *Australian Journal of Zoology* 42: 745–760.

Clark, E., 1936. The freshwater and land crayfishes of Australia. *Memoirs of the National Museum of Victoria* 10: 5–58.

Clark, E., 1937. The life history of the Gippsland crayfish. *The Australian Museum Magazine* 6: 186–192.

Clark, E., 1941. Revision of the genus *Euastacus* (crayfishes, family Parastacidae), with notes on the distribution of certain species. *Memoirs of the National Museum of Victoria* 12: 7–30.

Felsenstein, J. 1982. Numerical methods for inferring evolutionary trees. *Quarterly Review of Biology* 57: 379–404.

Hamr, P., 1992. A revision of the Tasmanian freshwater crayfish genus *Astacopsis* Huxley (Decapoda: Parastacidae). *Papers and Proceedings of the Royal Society of Tasmania* 126: 91–94.

Hedgecock, D., Tracey, M. L. and Nelson, K., 1982. Genetics. Pp. 285–403 in:. Abele, L.G. (ed.), *The biology of Crustacea. Volume 2, embryology, morphology and genetics.* Academic Press: New York.

Honan, J.A. and Mitchell, B.D., 1995. Reproduction of *Euastacus bispinosus* Clark (Decapoda: Parastacidae), and trends in reproductive characteristics of freshwater crayfish. *Journal of Marine and Freshwater Research* 46: 485–499.

Horwitz, P., 1988. Sea level fluctuations and the distributions of some freshwater crayfishes of the genus *Engaeus* (Decapoda: Parastacidae) in the Bass Strait area. *Australian Journal of Marine and Freshwater Research* 39: 427–502.

Horwitz, P., 1990a. *The conservation status of Australian freshwater Crustacea: with a provisional list of threatened species, habitats and potentially threatening processes.* Report Series No.14. Australian National Parks and Wildlife Service: Canberra.

Horwitz, P., 1990b. A taxonomic revision of species in the freshwater crayfish genus *Engaeus* Erichson (Decapoda : Parastacidae). *Invertebrate Taxonomy* 4: 427–614.

Horwitz, P., 1995. *A preliminary key to the species of Decapoda (Crustacea: Malacostraca) found in Australian inland waters.* Identification Guide No. 5. Co-operative Research Centre for Freshwater Ecology: Albury.

Horwitz, P., Adams, M. and Baverstock, P., 1990. Electrophoretic contributions to the systematics of freshwater crayfish genus *Engaeus* Erichson (Decapoda: Parastacidae). *Invertebrate Taxonomy* 4: 615–641.

Lintermanns, M. and Rutzou, T., 1991. *The status, distribution and management of the Murray crayfish* Euastacus armatus *in the Australian Capital Territory.* ACT. Research Report 6. Department of Environment, Lands and Planning, ACT Parks and Conservation Service.

Mayr, E., 1963. *Animal species and evolution.* Harvard University Press: Cambridge.

Morgan, G. J., 1983. *A taxonomic revision of the freshwater crayfish genus* Euastacus *Clark (Decapoda: Parastacidae).* Unpublished Ph. D. Thesis, Monash University.

Morgan, G.J., 1986. Freshwater crayfish of the genus *Euastacus* Clark (Decapoda: Parastacidae) from Victoria. *Memoirs of the Museum of Victoria* 47: 1–57.

Morgan, G.J., 1988. Freshwater crayfish of the genus *Euastacus* Clark (Decapoda: Parastacidae) from Queensland. *Memoirs of the Museum of Victoria* 49: 1–49.

Morgan, G.J., 1989. Two new species of the freshwater crayfish *Euastacus* Clark (Decapoda: Parastacidae) from isolated high country of Queensland. *Memoirs of the Queensland Museum* 27: 555–562.

Mulley, J.C. and Latter, B.D.H., 1980. Genetic variation and evolutionary relationships within a group of 13 species of penaeid prawns. *Evolution* 34: 904–916.

Olszewski, P., 1980. *A salute to the humble yabby.* Angus and Robertson: Sydney.

Patak, A. and Baldwin, J., 1994. Electrophoretic and immunochemical comparisons of haemocyanins from Australian fresh-water crayfish (Family Parastacidae): Phylogenetic implications. *Journal of Crustacean Biology* 4: 528–535.

Nei, M., 1978. Estimates of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583–590.

Nelson, K. and Hedgecock, D., 1980. Enzyme polymorphism and adaptive strategy in the decapod crustacea. *American Naturalist* 116: 238–280.

Rick, E.F., 1956. Additions to the Australian freshwater crayfish. *Records of the Australian Museum* 24: 1–6.

Riek, E.F., 1969. The Australian freshwater crayfish (Crustacea: Decapoda: Parastacidae). With description of new species. *Australian Journal of Zoology* 17: 855–918.

Richardson, B.J., Baverstock, P.R. and Adams, M., 1986. *Allozyme electrophoresis. A handbook for animal systematics and population studies.* Academic Press: Sydney.

Sneath, P.H.A. and Sokal, R.R., 1973. *Numerical taxonomy: the principles and practice of numerical classification.* Freeman: San Francisco.

Swain, R., Richardson, A.M.M. and Hortle, M., 1982. Revision of the Tasmanian freshwater crayfish genus *Astacopsis* Huxley (Decapoda: Parastacidae). *Australian Journal of Marine and Freshwater Research* 33: 699–709.

Swofford, D.L. and Selander, R.B., 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity* 72: 281–283.

Thorpe, J.P., 1982. The molecular clock hypothesis: biochemical evolution, genetic differentiation

and systematic. *Annual Review of Ecology and Systematics* 13: 139–168.

Tracey, M.L., Nelson, K., Hedgecock, D., Shleser, R.A. and Pressick, M.L., 1975. Biochemical genetics of lobsters: genetic variation and the structured of American lobster (*Homarus americanus*) populations. *Journal of the Fisheries Research Board of Canada* 32: 2091–2101.

Zeidler, W. and Adams, M., 1990. Revision of the Australian crustacean genus of freshwater crayfish *Gramastacus* Rick (Decapoda: Parastacidae). *Invertebrate Taxonomy* 3: 913–924.